Celecoxib Has a Positive Effect on the Overall Metabolism of Hyaluronan and Proteoglycans in Human Osteoarthritic Cartilage

HAFIDA EL HAJJAJI, ANNETTE MARCELIS, JEAN-PIERRE DEVOGELAER, and DANIEL-HENRI MANICOURT

ABSTRACT. ***Objective.*** To assess the effects of celecoxib, a cyclooxygenase (COX-2) selective inhibitor, on the metabolism of hyaluronan (HA) and proteoglycans (PG) in human cartilage explants with midrange severity of osteoarthritis (OA). Results were compared with those of diclofenac, a non-selective COX inhibitor.

Methods. Cartilage specimens (OA grade 4-8 on Mankin's scale) were pulsed with ^{3}H-glucosamine and chased in the absence or presence of 1-10 µg/ml of celecoxib or diclofenac. After papain digestion, the labeled chondroitin sulfate and HA molecules were purified by anion-exchange chromatography.

Results. Diclofenac did not affect the metabolic balance of PG and HA whereas, in a relatively dose-dependent manner, celecoxib increased the synthesis of HA and PG; celecoxib also reduced the net loss of labeled HA and PG molecules from cartilage explants.

Conclusion. In short term *in vitro* cultures, celecoxib has a favorable effect on the overall metabolism of PG and HA. It is therefore unlikely that this drug would have a detrimental effect on articular cartilage during longterm administration. Further, celecoxib might help counteract the depletion of HA seen in OA cartilage. (J Rheumatol 2003;30:2444–51)

Key Indexing Terms:
CARTILAGE CELECOXIB DICLOFENAC PROTEOGLYCANS HYALURONAN

Articular cartilage is a metabolically active tissue. The chondrocytes maintain a balance between the rate of degradation of the different matrix components and the rate of synthesis and incorporation of these molecules. On the other hand, the extracellular matrix protects the cells from the potentially damaging biomechanical forces generated by joint loading and motion. In osteoarthritic (OA) cartilage, there is an imbalance of these rates, which ultimately results in a progressive disruption of the matrix and loss of matrix components.

The most abundant molecules of articular cartilage matrix are collagen and proteoglycans (PG). By binding many polyanionic PG monomers non-covalently, a single filamentous molecule of hyaluronan (HA) forms supramolecular aggregates of very large size which can then be firmly entrapped at high concentration within the collagenous meshwork and in so doing, provide cartilage with its elasticity and stiffness on compression[1]. Therefore, any decrease in the cartilage concentration of PG and HA, as occurs in OA and other arthritides, compromises the functional properties of the articular tissue.

Nonsteroidal antiinflammatory drugs (NSAID) are widely prescribed to patients with inflammatory joint diseases and their therapeutic effect results, at least in part, from their ability to inhibit cyclooxygenase (COX), and hence the local production of prostaglandins, which are potent mediators of the inflammatory reaction[2]. Two isoforms of COX have been identified and characterized: COX-1, which is constitutively expressed in most tissues, and COX-2, which is inducible in response to proinflammatory cytokines and mitogens[3]. It is generally believed that the beneficial effects of NSAID are related to their ability to inhibit COX-2 and that their renal and gastrointestinal (GI) toxicity results from their inhibition of COX-1, a contention that has led to the development of highly selective COX-2 inhibitors[4]. It should however be stressed that COX-1 derived prostaglandins can contribute to the inflammatory response[5] and that COX-2 derived prostaglandins perform physiologically important roles such as the maintenance of normal renal function[6] and the protection of the GI tract from injury[7,8].

Although NSAID produce relief of pain and improvement of joint mobility in patients with arthritides, it is

From the ICP Christian de Duve Institute of Cellular Pathology and the Department of Rheumatology, Saint-Luc University Hospital, Catholic University of Louvain, Brussels, Belgium.

Supported by Grant 3.4597.02 of the Fonds de la Recherche Scientifique Médicale (Belgium).

H. El Hajjaji, Doctoral Student; A. Marcelis, Laboratory Technician, ICP Christian de Duve Institute of Cellular Pathology; J-P. Devogelaer, Professor of Medicine, Department of Rheumatology, Saint-Luc University Hospital; D-H. Manicourt, Professor of Medicine, Head of the Connective Tissue Unit, ICP Christian de Duve Institute of Cellular Pathology and Department of Rheumatology, Saint-Luc University Hospital.

Address reprint requests to Dr. D-H. Manicourt, Department of Rheumatology, UCL 5390, Avenue Mounier, 1200 Brussels, Belgium.

Submitted September 17, 2002; revision accepted March 26, 2003.

important to assess the direct effects of such compounds on articular cartilage metabolism. This point is most relevant to clinical practice since it is essential that compounds used to treat joint disorders do not impair the ability of the chondrocyte to repair its already damaged matrix. Accordingly, *in vitro* and *in vivo* investigations conducted with non-selective and moderately selective COX-2 inhibitors have shown that some NSAID inhibit the synthesis of cartilage PG whereas others do not[9-14]. Further, 2 recent studies suggest that selective COX-2 inhibitors may help to prevent the loss of cartilage PG induced by interleukin-1[15,16]. On the other hand, it is somewhat surprising that, so far, few investigative efforts have been devoted to the effect of NSAID on the metabolism of HA[12,14]. Indeed, this glycosaminoglycan plays a central role in the supramolecular organization of PG aggregates, and hence on the biomechanical properties of articular cartilage. Further, there is a dramatic reduction in the HA content of cartilage in experimental OA[17] and in human OA[18,19], and this in contrast to ageing[20,21].

Accordingly, we investigated the effect of celecoxib on the overall metabolism of HA and PG in human OA cartilage explants. This recently marketed COX-2 specific inhibitor shows sustained antiinflammatory and analgesic activity as well as good tolerability in therapy for rheumatic diseases[4]. Results were compared to those obtained with diclofenac, a non-selective COX-2 inhibitor, which is usually used as a reference NSAID in clinical trials[4].

MATERIALS AND METHODS

Materials. Celecoxib was a generous gift from Pharmacia (Brussels Belgium). Culture plates and media were from Invitrogen/Gibco BRL (Merelbeke, Belgium). ITS premix was from ICN (sAsse, Belgium) and [^{3}H]-glucosamine from Amersham Pharmacia Biotech (Roosendaal, The Netherlands). Dialysis membranes (molecular weight cut-off: 3500) were from Spectrum (Los Angeles, CA, USA) and Econo-Pac Q cartridges were from Bio-Rad (Nazareth, Belgium). All other reagents were from Sigma-Aldrich (Bornem, Belgium).

Cartilage sampling and explant culture. Human knee joints were collected within 2 h of surgery from patients undergoing total knee arthroplasty for OA. During the week preceding surgery, patients were allowed to take paracetamol or dextropropoxyphen H Cl, but no NSAID.

Immediately after surgery, full thickness cartilage was aseptically removed from the medial femoral condyle of each donor and washed with sterile phosphate buffered saline containing antibiotics (100 units/ml penicillin and 100 units/ml streptomycin). Three to four slices were taken at random and processed for the histological-histochemical grading system devised by Mankin, *et al*[22] whereas the other cartilage specimens were cut into small pieces in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin and streptomycin. The tissue was washed several times with this medium and aspirated free from liquid. Cartilage pieces were then weighed and distributed into the different wells of multi-well culture plates (typically between 30-60 mg tissue/well). DMEM supplemented with antibiotics and 20% (v/v) fetal calf serum was added to each well (1 ml/50 mg tissue) and the culture plates were incubated at 37°C in 5% CO_2 in air for 48 h before conducting the pulse and chase studies.

As the overall metabolism of articular cartilage can change markedly with the degree of severity of the disease process[23], we only included patients having a histological-histochemical grading system ranging from 4 to 8, i.e., being in the midrange of severity of the osteoarthritic disease process. A total of 24 patients (16 women and 8 men) were therefore included in the study. Since the total amount of cartilage obtained for each patient was insufficient to conduct the pulse and chase studies in the presence of different concentrations of NSAID, the patients were randomly distributed into 2 groups of 12 each. Pulse studies were carried out in one group (median age: 59 years; range: 51-64) and chase studies in the other group (median age: 60 years; range: 54-66).

For each pulse and chase study, the cartilage from one individual was used and tissue cultures were conducted in triplicate: that is, for the control culture as well for each NSAID concentration, 3 cartilage explants were cultured separately. Reported values are the means of the triplicate cultures.

Pulse studies. After 2 days of culture, the culture medium was aspirated and the explants were washed 3 times with DMEM. Explants were resuspended in DMEM (1 ml/50 mg tissue) supplemented with antibiotics, 4% (w/v) bovine serum albumin (BSA), ITS premix, and [^{3}H]-glucosamine (50 µCi/ml). To each well, a solution of NSAID dissolved in dimethylsulfoxide (DMSO) was added (10 µl/ml culture medium) to achieve a final concentration of 1 to 10 µg/ml. Control cultures received 10 µl/ml of DMSO that was free of any NSAID. Culture wells were then incubated for 12 h.

Chase studies. After 2 days of culture, the cartilage pieces were aspirated free of medium, washed 3 times with DMEM and incubated for 12 h in DMEM (1 ml/50 mg tissue) supplemented with antibiotics, BSA (4% w/v), ITS premix, and [^{3}H]-glucosamine (50 µCi/ml). After pulse labeling, the cartilage pieces were washed 3 times with DMEM and resuspended in DMEM supplemented with ITS, BSA, and antibiotics. To each well, a solution of NSAID dissolved in DMSO was added (10 µl/ml culture medium) to achieve a final concentration of 1 to 10 µg/ml. Control cultures received 10 µl/ml of DMSO that was free of any NSAID. Culture wells were then incubated for 24 h.

Isolation and purification of PG and HA. At the end of the pulse labeling and non-radioactive chase periods, the culture media were removed and the cartilage pieces were washed several times. Media and corresponding washes were then combined, enriched in bovine nasal PG monomers (500 µg/ml) and HA (10 µg/ml) before being dialyzed against 0.15 M sodium chloride, 0.05 M sodium acetate, and 5 mM EDTA, pH 6.0. Papain (10 µg/ml) and cysteine HCl (20 mM) were added to each sample, which was incubated for 24 h at 60°C. Cartilage specimens were lyophilized to obtain their dry weight and then digested with papain (100 µg/ml) for 24 h at 60°C. Digests of cartilage and media were aliquotted and samples were subjected either to ion-exchange chromatography or to biochemical determinations.

Papain digests were purified by chromatography on an Econo-Pac Q column as described[12,14]. Typically, a first [^{3}H]-radiolabeled peak was eluted at about 0.2–0.3 M NaCl and a second radiolabeled peak at about 1 M NaCL. Fifty to seventy percent of the material present in the first peak was identified as [^{3}H]-HA as it was sensitive to Streptomyces hyaluronidase. The material present in the second peak was resistant to digestion with Streptomyces hyaluronidase but about 90% of its content was digested by chondroitinase ABC. This second peak was therefore identified as chondroitin sulfate and hence as [^{3}H]-PG. The 10% of the material that was present in the second peak and that was resistant to digestion with both Streptomyces hyaluronidase and chondroitinase ABC is likely to be keratan sulfate, another component of PG molecules, although digestion with keratanase was not carried out in the present study.

The rates of biosynthesis of HA and PG were determined by the summation of [^{3}H]-HA and [^{3}H]-PG disintegrations per min (dpm) found in papain-digested tissues and media at the end of the 12 h pulse period. Since cartilage specimens cultured over 72 h had lost less than 5% of their original hydroxyproline content into the medium, results were expressed as dpm/h/mg of hydroxyproline.

At the end of the 24-h nonradioactive chase period, the [^{3}H]-HA and [^{3}H]-PG dpm found in the media and corresponding papain-digested tissue specimens wee summed to yield the total incorporated [^{3}H]-HA and [^{3}H]-PG dpm. The chase medium contains not only degraded glycosaminogly-

cans but also intact HA and PG molecules that were being synthesized at the end of the pulse labeling period; and that were not incorporated into the matrix and lost into the medium during the subsequent nonradioactive chase period; the radiolabeled material recovered in the medium during the 24-h chase period was therefore described as net loss rather than catabolism. This net loss was expressed as the percentage of total incorporated dpm found in the medium samples of the 24-h nonradioactive chase period.

Biochemical determinations. Hydroxyproline was determined by the method of Woessner[24] and hexuronate by the method of Bitter and Muir[25]. HA was quantified by a specific enzyme-linked immunosorbent assay[26].

Statistical analysis. The statistical significance of the differences observed between groups was evaluated by the Mann-Whitney U test. In each group, the significance of the differences in HA and PG metabolism observed in the presence of different NSAID concentrations were evaluated by the Wilcoxon signed rank test. P values < 0.05 were considered statistically significant.

RESULTS

Biochemical and metabolic characterization of cartilage explants. In the group of cartilage explants used for pulse studies (group A), the median content of hydroxyproline amounted to 69 µg/mg tissue dry weight (range: 60–80), and a similar value of 70 µg/mg tissue dry weight (range: 61–78) was found for the median content of hydroxyproline in cartilage specimens used for chase experiments (group B). Further, as shown in upper panels of Figure 1, the median content of hexuronate, and thus PG, was also similar in both groups; there was no statistically significant difference in the median content of HA ($U = 41$, $p = 0.529$) between the 2 groups.

In the explants incubated in the absence of NSAID, the rates of PG and HA synthesis (Figure 1, middle panels) were distributed over a wide range of values. Likewise, when radiolabeled cartilage explants were cultured in the absence of drug, the net loss of [^{3}H]-HA and [^{3}H]-PG molecules was also distributed over a wide range of values (Figure 1, lower panels). Obviously, these wide variations in metabolism that were exhibited by the cartilage explants cultured in the absence of drug were likely to hamper the assessment of the effects of NSAID on PG and HA metabolism. Therefore, the rates of proteoglycan and HA metabolism that were observed for cartilage explants bathed with different concentrations of NSAID were divided by the values obtained for cartilage specimens incubated in the absence of drug to yield percentage changes.

Effects of celecoxib and diclofenac on the synthesis of PG and HA. At the concentrations of 1 to 6 µg/ml (Figure 2, right panel A), diclofenac did not significantly change the total amounts of [^{3}H]-PG molecules that were recovered from the tissue and medium, and this is in contrast to celecoxib, which enhanced PG synthesis in a dose-dependent manner (Figure 2, left panel A).

Celecoxib also enhanced in a relatively dose-dependent manner the total amounts of newly synthesized HA molecules over the range of concentrations of 1–6 µg/ml (Figure 3, left panel A) but no further increase in HA synthesis was observed at a concentration of 10 µg/ml. In the range of concentrations studied however, diclofenac was unable to increase the total amounts of [^{3}H]-HA molecules (Figure 3, right panel A).

During the pulse studies conducted in the absence of drug, a relative proportion of newly synthesized [^{3}H]-PG and [^{3}H]-HA molecules were not incorporated into the matrix and were lost into the culture medium. Further, this relative loss of radiolabeled molecules varied from one donor to another. Therefore, in each experiment, the amounts of [^{3}H]-PG and [^{3}H]-HA (as expressed in dpm/h/mg of hydroxyproline) found in the explants after pulse-labeling in the presence of different concentrations of NSAID were divided by the amounts observed in the control explants pulse-labeled in the absence of drug to yield percent changes.

The changes in the tissue content of [^{3}H]-PG obtained at each NSAID concentration are shown in the B panels of Figure 2. The relative amounts of [^{3}H]-PG molecules remaining in cartilage matrix were unaffected by diclofenac over the range of concentrations studied whereas a significant increase was observed with celecoxib at a concentration of 6 µg/ml. No further increase was seen at a celecoxib concentration of 10 µg/ml.

The changes in the tissue content of [^{3}H]-HA observed at each NSAID concentration are shown in the B panels of Figure 3. At a concentration of 3 µg/ml, celecoxib had already produced a significant increase in the tissue content of [^{3}H]-HA. The tissue content of labeled HA molecules increased further with a celecoxib concentration of 6 µg/ml and then reached a plateau at a celecoxib concentration of up to 10 µg/ml. On the other hand, in the range of concentrations of 1–6 µg/ml, diclofenac was unable to significantly change the amount of [^{3}H]-HA molecules remaining within the matrix of cartilage specimens.

Effects of celecoxib and diclofenac on the net loss of PG and HA. Changes observed for the net loss of [^{3}H]-PG molecules from cartilage explants during the 24-h nonradioactive chase period are given in the upper panels of Figure 4. The net loss was unaffected by diclofenac at concentrations of 1, 3, and 6 µg/ml, whereas in explants bathed with celecoxib a marked inhibition of the net loss of [^{3}H]-PG was noted at a concentration of 1 µg/ml ($p = 0.004$) and this inhibition became stronger at celecoxib concentrations of 3 and 6 µg/ml.

Celecoxib also reduced the net loss of [^{3}H]-HA molecules in a dose-dependent manner (Figure 4, lower left panel). The reduction was statistically significant at a celecoxib concentration of 1 µg/ml ($p = 0.002$) and the strength of the reduction increased over the range of concentrations of 3 to 6 µg/ml. No further reduction could be observed at a celecoxib concentration of 10 µg/ml. In contrast, at the 3 concentrations tested (lower right panel), diclofenac did not induce any significant change in the net loss of radiolabeled HA molecules from cartilage explants during the 24-h chase period.

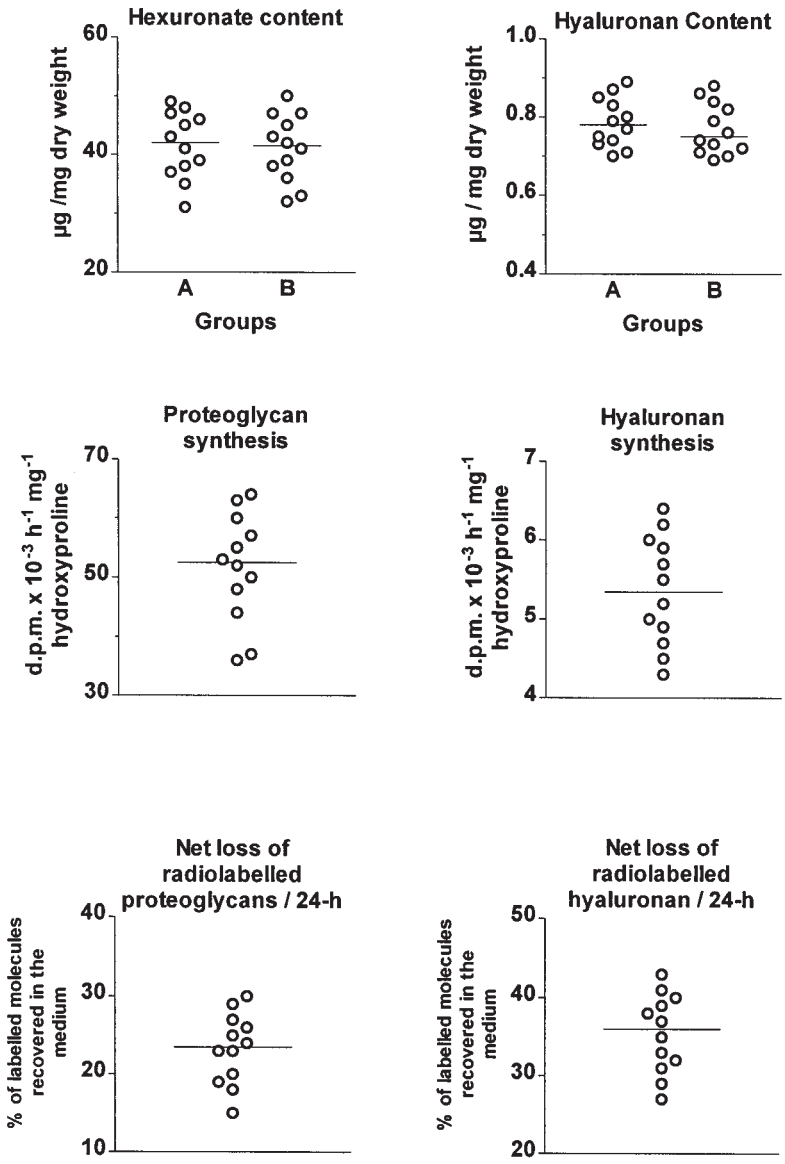

Figure 1. Distribution of values of total proteoglycan (Hexuronate) content, total hyaluronan content, and rates of proteoglycan and hyaluronan synthesis during a 12-h pulse period, and net loss of newly synthesized proteoglycan and hyaluronan molecules over a 24-h nonradioactive chase period in cartilage explants that were cultured in the absence of nonsteroidal antiinflammatory drugs. Explants of group A were used for pulse experiments and explants of group B were used for chase studies. In each column scatter, the horizontal line corresponds to the median value.

DISCUSSION

Our results show that celecoxib not only enhances the rate of HA synthesis but also reduces the net loss of newly synthesized HA molecules in human cartilage explants with OA in a dose-dependent manner.

Although one should exercise much caution in extrapolating the results obtained *in vitro* over short periods to *in vivo* situations, there is some evidence that changes in cartilage metabolism observed *in vitro* might also occur *in vivo*. Indeed, Dingle[13] has measured the rate of PG synthesis in OA cartilage explants obtained from patients who were taking NSAID on a regular basis up to the day of surgery. The action of the drug on PG synthesis was classified as either stimulatory or inhibitory according to previous *in vitro* studies. The author was able to confirm the previously reported *in vitro* effect of a given drug when the rate of PG synthesis was assessed immediately after cartilage tissue sampling, but no longer after a 5-day washout time period.

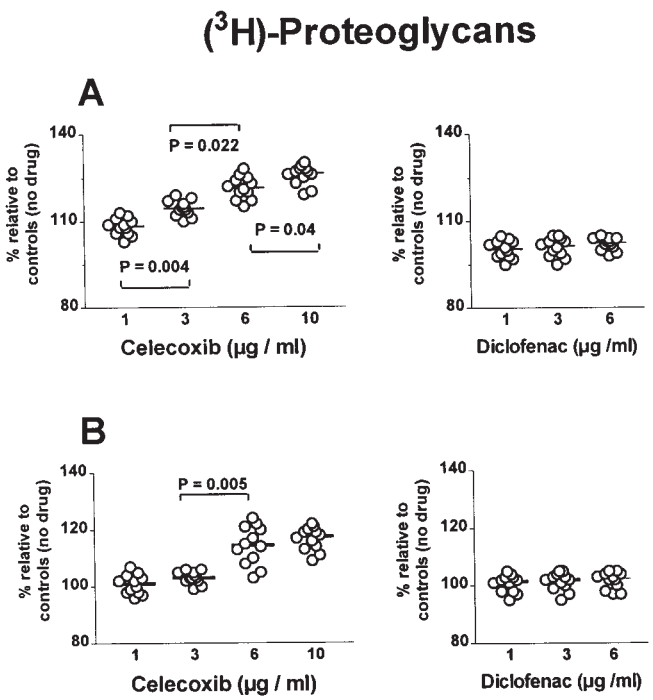

Figure 2. Effects of different concentrations of celecoxib and diclofenac on the total amounts (tissue and medium) of newly synthesized proteoglycan molecules (A panels). The effects of the drugs on the relative amounts of newly synthesized proteoglycans remaining in cartilage matrix at the end of the pulse period are illustrated in B panels. Results are expressed as the relative percentage (%) of values observed in corresponding explants cultured in the absence of drug. In each column scatter, the horizontal line corresponds to the median value. p comparison by the Wilcoxon signed-rank test.

These results are further strengthened by the observation that, in OA cartilage explants obtained from patients who were untreated prior to surgery, the rate of PG synthesis did not exhibit change significantly when assessed either immediately after cartilage sampling or after the washout period. On the other hand, several clinical studies[10,27,28] have suggested that some NSAID might accelerate joint destruction in human OA, although their methodological approaches were flawed.

The concentrations of free drugs present *in vitro* were likely to be close to the concentrations of free drug present *in vivo* since, in our culture system, the concentration of albumin was similar to that found in synovial fluid and the total concentrations of celecoxib and diclofenac were similar to the range of concentrations found for these drugs in human body fluids[29,30]. On the other hand, to restrict the topographical and disease-related variations in cartilage composition and metabolism[23], sampling of full thickness cartilage was limited to the medial femoral condyle and we explored only cartilage specimens in the mid-range of severity of OA, i.e., at a stage of the disease process in which many patients consult physicians for joint pain and start receiving NSAID.

In contrast to diclofenac, celecoxib had a positive effect on the metabolic balance of HA. This finding is worth stressing since, although HA is present in low concentrations within cartilage matrix, this glycosaminoglycan is the central core of PG aggregates, a supramolecular organization that dramatically increases the rheological properties of PG molecules and, in so doing, affects the dynamic behavior of cartilage in compression[31]. Furthermore, the progressive reduction in HA content of cartilage seen in experimental and human OA[17,18] is likely to contribute to the apparent irreversibility of the OA disease process[32] and contrasts with the age-related increase in the HA content of normal articular cartilage[19,20].

Although the effects of celecoxib on HA metabolism might be of great biological and therapeutic significance in OA, the exact mechanisms of action of this drug are not clear. Very little is known about the mechanisms regulating the expression and/or activity of the 2 HA synthases present at the cell surface of chondrocytes[33]. Further, since no hyaluronidase active at the near neutral pH of cartilage matrix has been identified, it has been suggested that degradation and loss of HA molecules may result from the action of oxygen-derived free radicals (ODFR)[34]. However, if ODFR played an important role in the loss of HA molecules from cartilage explants, then one might have expected that diclofenac would reduce the net loss of newly synthesized HA molecules from cartilage matrix since NSAID have been reported to block the production of ODFR[35].

Celecoxib was also able to enhance PG synthesis and to reduce the loss of newly synthesized PG molecules from OA cartilage explants and this in contrast to a recent report showing that celecoxib had no significant effect on PG metabolism in healthy human cartilage[16]. Such difference between normal and OA cartilage in the metabolic response to drugs has already been observed with other NSAID and is apparently related to a greater diffusion of the drug throughout the matrix of OA cartilage[10,36]. Indeed, although NSAID may bind differentially to cartilage matrix, there is an inverse relationship between, on the one hand, the decrease in the matrix concentration of negatively charged PG molecules, which is proportional to the gravity of the OA disease process, and, on the other hand, the uptake of the drug by the cartilage tissue.

The positive effect of celecoxib on PG metabolism contrasts with the neutral effect of diclofenac. Such a difference between drugs in the metabolic response of cartilage has already been observed in previous *in vivo* and *in vitro* studies showing that some drugs stimulate the rate of PG synthesis by cartilage whereas others either inhibit PG synthesis or have no effect[10,12,13]. Although aspirin, and to a lesser extent, ibuprofen, can inhibit PG synthesis by inhibiting glucuronyltransferase, an enzyme involved in the elongation of chondroitin sulfate chains in the nascent PG molecule[37], the mechanisms responsible for these marked

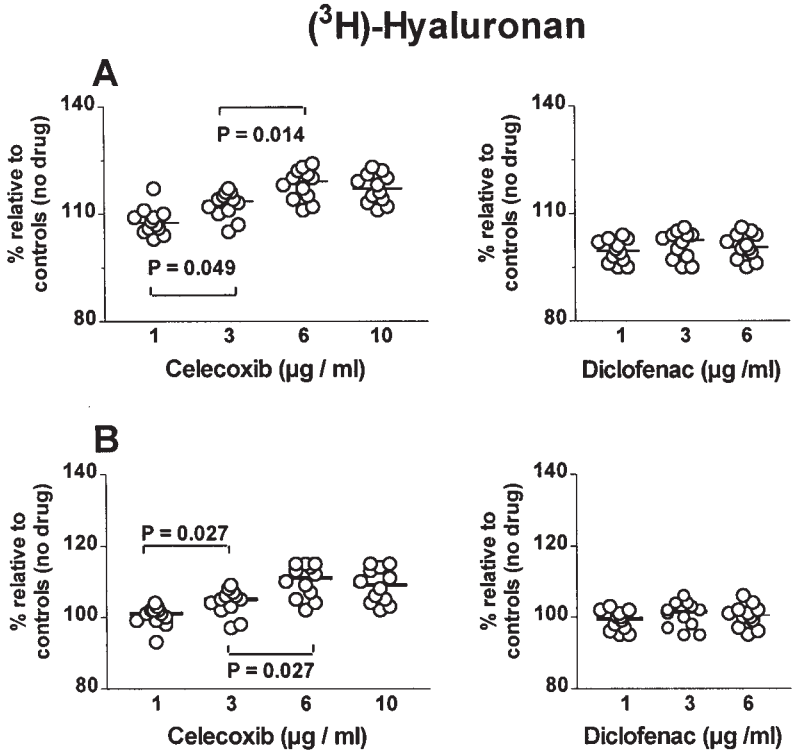

Figure 3. Effects of different concentrations of celecoxib and diclofenac on the total amounts (tissue and medium) of newly synthesized hyaluronan molecules (A panels). The effects of the drugs on the relative amounts of newly synthesized hyaluronan remaining in cartilage matrix at the end of the pulse period are illustrated in the B panels. Results are expressed as the relative percentage (%) of values observed in corresponding explants cultured in the absence of drug. In each column scatter, the horizontal line corresponds to the median value. p comparison by the Wilcoxon signed-rank test.

differences in the effect of NSAID on PG synthesis remain to be elucidated and are apparently not related to the chemical class of the drug or to its capacity to inhibit PG production[10,38]. On the other hand, in a recent study conducted with healthy human cartilage, celecoxib was able to reverse the inhibition of PG synthesis induced by interleukin-1 and tumor necrosis factor alpha[16].

The reduction in the net loss of PG produced by celecoxib might be, at least in part, related to the positive effect of this drug on the overall metabolism of HA. Indeed, by limiting PG aggregation, any decrease in the HA content of cartilage is likely to favor the loss of newly synthesized PG molecules by diffusion and/or by proteolytic degradation[39].

On the other hand, it is generally accepted that the major enzymes involved in the turnover of PG, both in health and disease, are aggrecanase and several members of the family of matrix metalloproteinases (MMP), including stromelysin-1 (MMP-3) and the 3 collagenases (MMP-1, -8, and -13)[40,41]. This contention is further strengthened by the observations that synthetic hydroxamate compounds known to inhibit the activity of aggrecanase and MMP have the ability to inhibit the release of PG from cartilage explants[41,42]. Accordingly, some NSAID might act by inhibiting the expression and/or activity of such enzymes. Indeed, naproxen has been reported to reduce the total amounts of latent and active MMP present in canine articular cartilage[11] whereas 4-hydroxy aceclofenac, a major metabolite of aceclofenac in humans, markedly inhibits the secretion of aggrecanase, MMP-1, and MMP-3 by chondrocytes[43], this effect being apparently unrelated to the reduction in the production of prostaglandin E_2 or to the increase in intracellular cyclic AMP concentrations. Further, other studies also suggest that NSAID could act either as reversible enzymatic inhibitors[44] or by inhibiting COX, which mediate the induction of membrane-type metalloproteinase-1 (MMP-14), an enzyme able to activate gelatinase A (MMP-2) and MMP-13[45]. Obviously, the effects of NSAID on PG metabolism are still poorly understood.

In conclusion, although celecoxib did not normalize the changes in the metabolism of OA cartilage, this drug had a favorable effect on the metabolism of both HA and PG and, accordingly, should not hamper the biomechanical properties of OA cartilage matrix. However, it remains to be established whether changes observed *in vitro* over short periods would also be observed *in vivo* over long periods.

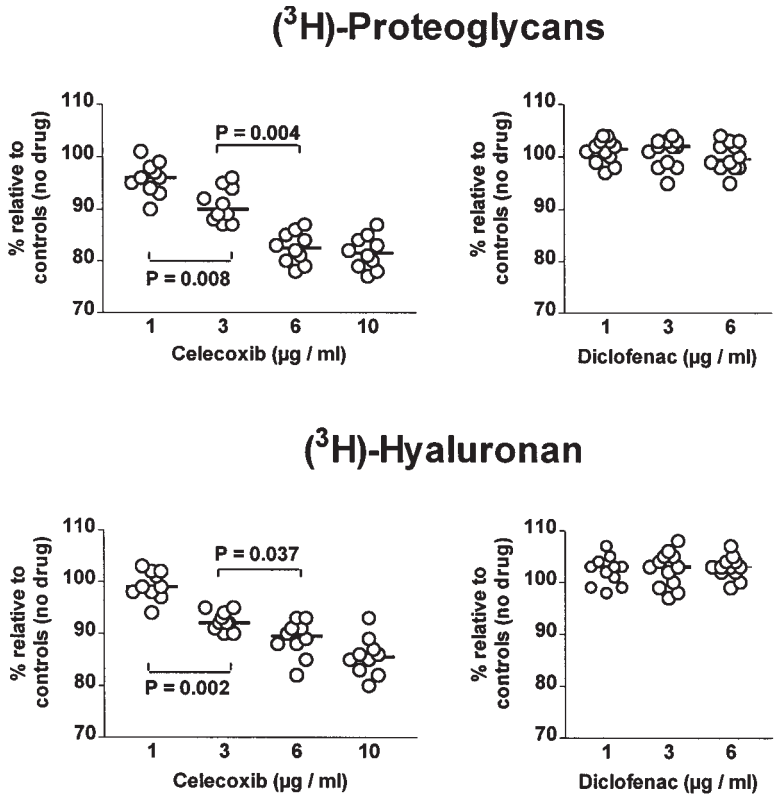

Figure 4. Effects of different concentrations of celecoxib and diclofenac on the net loss of newly synthesized proteoglycan and hyaluronan molecules from cartilage explants during the 24-h nonradioactive chase period. Results are expressed as the relative percentage (%) of values observed in corresponding explants cultured in the absence of drug. In each column scatter, the horizontal line corresponds to the median value. p comparison by the Wilcoxon signed-rank test.

ACKNOWLEDGMENT

The authors thank Drs. N. Kormoss and M. Lopinot for their support.

REFERENCES

1. Hardingham T. Proteoglycans and glycosaminoglycans. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. San Diego: Academic Press; 1999:71-81.
2. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 1971;231:232-5.
3. Smith WL, Garavito RM, Dewitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 1996;271:33157-60.
4. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis. Proc Natl Acad Sci USA 1999;96:7563-8.
5. Gilroy, DW, Tomlinson A, Willoughby DA. Differential effects of inhibitors of cyclooxygenase (cyclooxygenase 1 and cyclooxygenase 2) in acute inflammation. Eur J Pharmacol 1998;355:211-7.
6. Dinchuk JE, Car BD, Focht RJ, et al. Renal abnormalities in the altered inflammatory response in mice lacking cyclooxygenase II. Nature 1995;378:406-9.
7. Gretzer B, Ehrlich K, Maricic N, Lambrecht N, Respondek M, Peskar BM. Selective cyclo-oxygenase-2 inhibitors and their influence on the protective effect of a mild irritant in the rat stomach. Br J Pharmacol 1998;123:927-35.
8. Schmassmann A, Peskar BM, Stettler C, Netzer P, Stroff FT, Flogerzi B, Halter F. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. Br J Pharmacol 1998;123:795-804.
9. Howell DS, Pita JC, Muller FJ, Manicourt DH, Altman RD. Treatment of OA with tiaprofenic acid: biochemical and histological protection against cartilage breakdown in the Pond-Nuki canine model. J Rheumatol 1991;18 Suppl 27:138-42.
10. Brandt KD. Should osteoarthritis be treated with nonsteroidal anti-inflammatory drugs? Rheum Dis Clin North Am 1993;19:697-712.
11. Ratcliffe A, Azzo W, Saed-Nejad F, Lane N, Rosenwasser MP, Mow VC. In vivo effects of naproxen on composition, proteoglycan metabolism, and matrix metalloproteinase activities in canine articular cartilage. J Orthop Surg 1993;11:163-71.
12. Manicourt D H, Druetz-Van Egeren A, Haazen L, Nagant de Deuxchaisnes C. Effects of tenoxicam and aspirin on the metabolism of proteoglycans and hyaluronan in normal and osteoarthritic human articular cartilage. Br J Pharmacol 1994;113:1113-20.
13. Dingle JT. Non-steroidal anti-inflammatory drug administration in

the treatment of osteoarthritis. In: Reginster J-Y, Pelletier J-P, Martel-Pelletier J, Henrotin Y, editors. Osteoarthritis. Clinical and experimental aspects. Berlin: Springer-Verlag; 1999:370-87.
14. Blot L, Marcelis A, Devogelaer JP, Manicourt DH. Effects of diclofenac, aceclofenac and meloxicam on the metabolism of proteoglycans and hyaluronan in osteoarthritic human articular cartilage. Br J Pharmacol 2000;131:1413-21.
15. Hardy MM, Seibert K, Manning PT, et al. Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritic explants. Arthritis Rheum 2002;46:1789-803.
16. Mastbergen SC, Lafeber FP, Bijlsma JW. Selective COX-2 inhibition prevents proinflammatory cytokine-induced cartilage damage. Rheumatology 2002;41:801-8.
17. Manicourt DH, Pita JC. Progressive depletion of hyaluronic acid in early experimental osteoarthritis in dogs. Arthritis Rheum 1988;31:538-44.
18. Sweet MB, Thonar EJ, Immelman AR, Solomon L. Biochemical changes in progressive osteoarthrosis. Ann Rheum Dis 1977;36:387-98.
19. Thonar EJ, Sweet MB, Immelman AR, Lyons G. Hyaluronate in articular cartilage: age-related changes. Calcif Tissue Res 1978;26:19-21.
20. Rizkalla G, Reiner A, Bogoch E, Poole AR. Studies of the articular cartilage proteoglycan aggrecan in health and osteoarthritis. Evidence of molecular heterogeneity and extensive molecular changes in disease. J Clin Invest 1992;90:2268-77.
21. Holmes MW, Bayliss MT, Muir H. Hyaluronic acid in human articular cartilage. Age-related changes in content and size. Biochem J 1988;250:435-41.
22. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg 1971;53A:523-37.
23. Muir H. Current and future trends in articular cartilage research and osteoarthritis. In: Kuettner K, Schleyerbach DVM, Hascall VC, editors. Articular cartilage biochemistry. New York: Raven Press; 1986:423-40.
24. Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing a small portion of this amino acid. Arch Biochem Biophys 1961;93:440-7.
25. Bitter T, Muir H. A modified uronic acid carbazole reaction. Anal Biochem 1962;4:330-4.
26. Li XQ, Thonar EJ-MA, Knudson W. Accumulation of hyaluronate in human lung carcinoma as measured by a new hyaluronate ELISA. Connect Tissue Res 1989;19:243-53.
27. Rashad S, Revell P, Hemingway A, Low F, Rainsford K, Walker F. Effect of non-steroidal anti-inflammatory drugs on the course of osteoarthritis. Lancet 1989;8662:519-22.
28. Huskisson EC, Berry H, Gishen P, Jubb RW, Whitehead J. Effects of anti-inflammatory drugs on the progression of osteoarthritis of the knee. LINK Study Group. Longitudinal investigation of nonsteroidal anti-inflammatory drugs in knee osteoarthritis. J Rheumatol 1995;22:1941-6.
29. Netter P, Bannwarth B, Royer-Morrot MJ. Recent findings on the pharmacokinetics of non-steroidal anti-inflammatory drugs in synovial fluid. Clin Pharmacokinet 1989;17:149-62.
30. Davies NM, McLachlan AJ, Day RO, Williams KM. Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclo-oxygenase-2 inhibitor. Clin Pharmacokinet 2000;38:225-42.
31. Hardingham TE, Muir H, Kwan MK, Lai WM, Mow VC. Viscoelastic properties of proteoglycan solutions with varying proportions present as aggregates. J Orthop Res 1987;5:36-46.
32. Pita JC, Muller FJ, Manicourt DH, Buckwalter JA, Ratcliffe A. Early matrix changes in experimental osteoarthritis and joint disuse atrophy. In: Kuettner K, Schleyerbach DVM, Peyron JG, Hascall VC, editors. Articular cartilage and osteoarthritis. New York: Raven Press; 1992:455-69.
33. Hiscock DR, Caterson B, Flannery CR. Expression of hyaluronan synthases in articular cartilage. Osteoarthritis Cartilage 2000;8:120-6.
34. Ng CK, Handley CJ, Preston BN, Robinson HC, Bolis S, Parker G. Effect of exogenous hyaluronan and hyaluronan oligosaccharides on hyaluronan and aggrecan synthesis and catabolism in adult articular cartilage explants. Arch Biochem Biophys 1995;316:596-606.
35. Minta JO, Williams MD. Some nonsteroidal antiinflammatory drugs inhibit the generation of superoxide anions by activated polymorphs by blocking ligand-receptor interactions. 1985;12:751-7.
36. Palmoski MJ, Brandt KD. Relationship between matrix proteoglycan content and the effects of salicylate and indomethacin on articular cartilage. Arthritis Rheum 1983;26:528-31.
37. Hugenberg ST, Brandt KD, Cole CA. Effects of salicylate, aspirin and ibuprofen on enzymes required by the chondrocyte for synthesis of chondroitin sulfate. J Rheumatol 1993;20:2128-33.
38. Palmoski MJ, Brandt KD. Effects of salicylate and indomethacin on glycosaminoglycan and prostaglandin E_2 synthesis in intact canine knee cartilage ex vivo. Arthritis Rheum 1984;27:398-403.
39. Heinegard D, Hascall VC. Aggregation of cartilage proteoglycans. III. Characteristics of the proteins isolated from trypsin digests of aggregates. J Biol Chem 1974;249:4250-6.
40. Lark MW, Bayne EK, Flanagan J, et al. Aggrecan degradation in human cartilage. Evidence for both matrix metalloproteinase and aggrecanase activity in normal, osteoarthritic, and rheumatoid joints. J Clin Invest 1997;100:93-106.
41. Tortorella MD, Burn TC, Pratta MA, et al. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of protein. Science 1999;284:1664-6.
42. Grobelny D, Poncz L, Galardy RE. Inhibition of human skin fibroblast collagenase, thermolysin, and Pseudomonas aeruginosa elastase by peptide hydroxamic acid. Biochemistry 1992;31:7152-4.
43. Akimoto H, Yamazaki R, Hashimoto S, Sato T, Ito A. 4-Hydroxy aceclofenac suppresses the interleukin-1 induced production of promatrix metalloproteinases and release of sulfated-glycosaminoglycans from rabbit articular chondrocytes. Eur. J Pharmacol 2000;401:429-36.
44. Barracchini A, Franceschini N, Amicosante G, et al. Can non-steroidal anti-inflammatory drugs act as metalloproteinase modulators? An in-vitro study of inhibition of collagenase activity. J Pharm Pharmacol 1998;50:1417-23.
45. Takahashi Y, Kawahara F, Noguchi M, et al. Activation of matrix metalloproteinase-2 in human breast cancer cells overexpressing cyclooxygenase-1 or 2. FEBS Lett 1999;460:145-8.